EXHIBIT 17

Gene Therapy for Cystic Fibrosis*

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Following the cloning of the cystic fibrosis (CF) gene, *in vitro* studies rapidly established the feasibility of gene therapy for this disease. Unlike *ex vivo* approaches that have been utilized for other genetic diseases such as adenosine deaminase deficiency, gene therapy for CF will likely require direct *in vivo* delivery of gene transfer vectors to the airways of patients with CF. Hence, major research efforts have been directed at the development of efficient gene transfer vectors that are safe for use in human subjects. Several vectors have now emerged from the laboratory for evaluation in clinical safety and efficacy trials in the United States and in the United Kingdom. Adenovirus-mediated gene transfer has been utilized for initial clinical safety and efficacy trials in the United States, while liposome-mediated gene transfer has been chosen for initial clinical safety and efficacy trials in the United Kingdom. The rationale and laboratory studies are reviewed leading to initial clinical safety and efficacy trials. Also reviewed are the currently available vectors for potential use in clinical studies, their advantages and disadvantages, and the promises and pitfalls of current gene therapy efforts for CF in the United States focusing on adenovirus vectors in current clinical trials. *(Chest 1995; 107:77S-83S)*

AAV=adeno-associated virus; Cl^-=chloride; CF=cystic fibrosis; CFTR=cystic fibrosis transmembrane conductance regulator; E1=early region gene one; E3=early region gene three; MOI=multiplicity of infection

Key words: adeno-associated virus vectors; adenovirus vectors; airway epithelia; cationic liposomes; cystic fibrosis; gene therapy/transfer; molecular conjugates; retrovirus vectors

Cystic fibrosis (CF) is an autosomal recessive disorder affecting 1 in 2,500 live white births.[1] The disease is characterized by abnormal salt and water transport that leads to abnormal airway secretions, impaired mucociliary clearance, chronic bacterial infection, bronchiectasis, and premature death.[1,2] A variety of epithelial tissues are affected in this disease, including airway, pancreatic, sweat ductal, and gastrointestinal epithelia.[1,2] However, lung disease is the major cause of morbidity and mortality in this disorder. Accordingly, initial gene therapy efforts have been directed toward lung disease.

The CF gene, which codes for the CF transmembrane conductance regulator (CFTR) protein, was cloned in 1989,[3,4] and since that time, more than 300 mutations have been reported. The most common mutation is a three base pair deletion leading to deletion of phenylalanine (F) at position 508 (ΔF508) of the gene. This mutation leads to abnormal intracellular processing and trafficking of the mutant CFTR, and ultimately to defective apical membrane chloride (Cl^-) conductance in epithelial cells affected by this disorder.[5] Several investigators have proved that the protein product of CFTR is a cyclic adenosine monophosphate (cAMP)-mediated Cl^- channel.[6-8]

Because CF is an autosomal recessive disorder, introduction of a normal copy of the gene into the host cell should result in restoration of normal Cl^- transport function. In studies published by Drumm and colleagues[9] and Rich and colleagues,[10] introduction of a normal copy of the CFTR gene into CF epithelial cells using retrovirus and vaccinia virus vectors restored normal Cl^- transport function to these cells. Subsequently, Olsen and colleagues[11] demonstrated persistence of normal Cl^- transport function in CF epithelial cells for up to 6 months in culture following retrovirus-mediated gene transfer. These studies demonstrating restoration of normal Cl^- transport following *in vitro* gene transfer of wild-type (*ie*, normal) CFTR have established the feasibility of gene therapy for CF.

Of the 46 human gene therapy protocols that had been reviewed by the Food Drug Administration by mid-July 1993, 38 of the protocols involved *ex vivo* approaches.[12] That is, cells are removed from an organ, placed in cell culture, genetically modified, usually with retrovirus vectors, enriched for the population of cells expressing the desired gene through the use of selectable markers, and then reinfused into the appropriate organ of the human body. This approach, although effective for disorders such as adenosine deaminase deficiency, will be virtually impossible to perform in CF in view of the dichotomously branching airways of the lung, which prohibit effective *in vivo* removal and reimplantation of airway epithelial cells. Therefore, gene therapy for CF will require direct *in vivo* delivery of vectors encoding wild-type CFTR to the airways of patients with CF. To be successful, this approach mandates the use of gene transfer vectors that are both efficient and safe for human use.

Current Gene Transfer Vectors

A variety of gene transfer vectors are potentially

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Table 1—*Current Gene Transfer Vectors for Cystic Fibrosis Lung Disease*

	Advantages	Disadvantages
Adenovirus	Infects nondividing cells Tropic for respiratory epithelia	Repetitive dosing required Dose-related immune response Possible risk of insertional mutagenesis
Adeno-associated virus	Naturally defective Nonpathogenic, integrates into host cell genome, infects nondividing cells	Small insert size Risk of insertional mutagenesis DNA rescue by by coinfection with wildtype-virus
DNA-molecular conjugates	Transfect nondividing cells Offer tissue specificity through receptor binding	Repetitive dosing required High doses may cause immune response
Liposomes	Nontoxic, nonviral Transfect nondividing cells	Repetitive dosing required
Retrovirus	Integrates into host cell genome	Requires dividing cells Risk of insertional mutagenesis

available for use in human clinical trials. These vectors can be broadly grouped into those that integrate or insert into the host cell genome (retroviruses and adeno-associated viruses [AAVs]) and those that function as extrachromosomal, or episomal, DNA in the nucleus leading to transient expression (molecular conjugates, liposomes, and adenoviruses). A list of vectors used in gene therapy research for CF, with their advantages and disadvantages, appears in Table 1, and they are described in more detail below.

Retrovirus Vectors

Retrovirus-mediated gene transfer is the method of gene transfer utilized in most currently approved human gene transfer trials.[13] While human immunodeficiency virus is the most commonly known retrovirus, most retroviruses are structurally similar. They are all RNA viruses that have two viral long terminal repeats that are important for cellular integration and also contain promoter elements, a packaging signal, and a series of structural genes, *gag*, *pol*, and *env*. The *gag* gene encodes a precursor polypeptide that is cleaved to yield the capsid protein. The *pol* gene encodes reverse transcriptase and an enzyme involved in proviral integration. The *env* gene encodes the precursor to the envelope glycoprotein. A retrovirus vector is constructed by deleting the *gag*, *pol*, and *env* genes using restriction enzymes and inserting or cloning desired cDNAs (genes) into the remaining retrovirus genome.[14] Exogenous promoters may also be included in the sequences of the inserted gene or alternatively, the viral 5' long terminal repeat may be used to drive transcription of the inserted gene. Because these retrovirus vectors have had their viral genes deleted, they are replication-defective.

When a wild-type retrovirus infects a cell, glycoproteins on the virus particle bind to a specific cell surface receptor, which results in fusion and emptying of the nucleocapsid containing the RNA and its reverse transcriptase into the cytoplasm of the host cell. The RNA is reverse transcribed to form a cDNA, the provirus, which is translocated to the nucleus, where it integrates into the host cell chromosomes. The integrated provirus, through the normal process of DNA transcription, encodes new viral proteins and new viral RNA, which are assembled at the cell surface into new virus particles. When a replication-defective retrovirus vector infects a cell, its RNA is also reverse transcribed to form the provirus, which integrates into the host cell genome. However, unlike wild-type viruses, the integrated provirus from a retrovirus vector does not produce viral proteins or viral RNA. Instead, it encodes the inserted gene.

Because the retrovirus vector genome integrates into the host cell DNA, it offers a possibility for long-term expression, and perhaps a "cure." However, this process of random integration is associated with a risk of insertional mutagenesis and tumor formation. Also, the low rates of epithelial cell proliferation in normal human airways have made retrovirus vectors unattractive in *in vivo* gene transfer to the lung in view of their requirement for dividing cells.[15] Although a recent report suggests that airway epithelial cells in patients with CF may have significantly higher rates of cell turnover than measured in airways of normal subjects,[16] the experience with *in vivo* retrovirus-mediated gene transfer to the lung has been disappointing.

Adeno-Associated Viruses

Adeno-associated virus is a naturally defective parvovirus that is nonpathogenic.[17,18] The two most common serotypes infecting humans are serotypes 2 and 3. These viruses require the presence of a helper virus in order to replicate or cause a lytic infection. When AAV infects a cell in the absence of a helper virus, it integrates into the host cell genome and

becomes latent. Upon a subsequent wild-type adenovirus or wild-type herpes virus infection, the AAV genome can be rescued or excised from the chromosome to yield a lytic infection.

The AAV genome is small with a size of approximately 4.7 kb (kilobase pairs). Vector construction involves removal of all except approximately 0.2 kb of the virus genome.[18] The remaining 0.2 kb consists of the inverted terminal repeats at the 5' and 3' ends of the molecule. These inverted terminal repeats are important in the hairpin-type replication that is known to occur with this vector and also important for integration into the host cell genome. The region deleted includes the viral *rep* and *cap* genes, which are responsible for virus replication and nucleocapsid formation, respectively, and create approximately 4.5 kb of room for a cDNA insert. In the case of CFTR, which has a coding region of approximately 4.5 kb, this leaves little room for the use of either exogenous or endogenous promoters. Hence, AAV vectors encoding CFTR may actually exceed the size of the wild-type genome, leading to inefficient packaging and subsequent difficulties with virus production. Despite this limitation, *in vitro* correction of the CF Cl^- permeability defect has been reported in CF airway epithelial cells using AAV vectors coding for wild-type and/or truncated CFTR cDNAs.[19,20]

A potential risk with the AAV vectors, given their ability to integrate into the host cell genome, is insertional mutagenesis. Unlike wild-type AAV, which integrates into specific sites on chromosome 19,[21] AAV vectors appear to integrate into multiple random sites.[22] Nevertheless, AAV-CFTR vectors are promising because they appear to infect and express in nondividing cells, perhaps existing temporarily in episomal form until DNA replication or repair occurs allowing integration.

DNA-Molecular Conjugates

When plasmid DNA is linked to polylysine and a receptor ligand, this forms a DNA-molecular conjugate. This molecular conjugate may then bind to a specific cell surface receptor and enter the cell by receptor-mediated endocytosis. Addition of an inactivated adenovirus to this molecular complex using an antibody bridge results in more efficient gene transfer *in vitro* by virtue of the ability of the adenovirus to escape the endolysosomal pathway and increase DNA delivery to the nucleus.[23] Molecular conjugates have been shown to be efficient in airway epithelia *in vitro*. Moreover, recent data by Ferkol and colleagues[24,25] suggest that molecular conjugates that target the polymeric IgA receptor can deliver reporter genes to the airway epithelia *in vitro* and also *in vivo* following intravenous administration.

DNA-molecular conjugates are attractive in view of the potential for targeting to specific tissues through the use of tissue-specific ligands. Moreover, these conjugates appear to be capable of infecting and expressing in quiescent cells. However, a major concern with DNA molecular conjugates is the large protein load that is delivered with the DNA. The immunologic considerations and effects of repetitive dosing with this transient expression vector have not been fully addressed.

Liposomes

Liposome-mediated gene transfer is a popular method by which to deliver DNA to cells. Perhaps even more common has been the use of liposomes to deliver encapsulated drugs to patients. The liposomes utilized in the pharmaceutical industry have largely been of the simple liposome or negatively charged variety. The use of negatively charged liposomes for gene transfer has been limited by a low efficiency of DNA encapsulation with subsequent low efficiency of gene transfer. The inclusion of fusion proteins and pH-sensitive lipids in the lipid bilayer of simple liposomes have improved gene transfer efficiency.[26] However, the use of cationic liposomes has brought liposome-mediated gene transfer to the forefront of gene therapy research.

Cationic liposomes are composed of cationic lipids such as N[1-(2,3-dioleoxy)propyl] N,N,N trimethylammonium (DOTMA), dimethyldioctadecyl-ammonium bromide (DDAB), or 3β[N-N',N'-dimethylamino ethane-carbamoyl] cholesterol (DC-cholesterol) mixed in varying molar ratios with the neutral phospholipid, dioleylphoshpatidylethanolamine (DOPE). In some instances, detergents and cholesterol may be added (as has been done with DC-cholesterol) to increase stability and possibly reduce *in vitro* toxicity. Cationic liposomes have generally been believed not to encapsulate DNA, although a recent publication suggests that encapsulation may occur at low DNA to liposome ratios.[27] Instead, negatively charged plasmid DNA binds to cationic liposomes to form a DNA-liposome complex. DNA-liposome complexes may enter cells through either fusion to the cell membrane or alternatively, via endocytosis. Current evidence suggests that endocytosis is the major route of uptake for DNA-liposome complexes.[26,28]

Liposome-mediated gene transfer has been shown to be efficient *in vitro*[26,28-31] and potentially *in vivo*[32-36] in a variety of cell types. It may also be efficacious *in vivo* in view of recent reports suggesting possible restoration of normal Cl^- transport in transgenic mouse models of CF.[37,38] These reports, although controversial due to differences in electro-

physiologic properties between murine and human airways, offer promising insights with regard to the potential role of liposome-mediated gene transfer for CF. Moreover, cationic liposomes have been associated with a low toxicity profile, making them very attractive as a gene transfer vector for human studies.[39-41] Currently, a clinical gene transfer safety and efficacy trial using liposome-mediated gene transfer is being performed in the nasal cavity of patients with CF in the United Kingdom. The issue of repetitive dosing with this transient expression vector system has not been addressed.

Adenovirus Vectors

Adenovirus virus vectors have anchored gene therapy efforts for CF in the United States. Adenoviruses are icosahedral DNA viruses in which human serotypes 2 and 5 provide the backbone for current adenovirus vectors. Wild-type adenoviruses have a 36-kb genome, which for cloning purposes is conveniently divided into 100 map units of 360 base pairs each. The genome contains a series of early genes that are responsible for virus replication and antigen presentation and surveillance, and a series of late genes that encode viral structural proteins.[42-44] Current vectors based on adenovirus serotype 5 have generally had the early region one (E1) and early region three (E3) genes deleted.[45] Deletion of the E1 region makes the virus vector replication defective, while deletion of the E3 region creates enough room to insert the CFTR gene with a suitable promoter to drive transcription. Current adenovirus vectors encoding CFTR based on serotype 2,[46] which is approximately 90% homologous to serotype 5, have only had the E1 region deleted such that vector genomes may actually exceed the size of the wild-type virus genome, leading to inefficient packaging or formation of viral particles and low infectious titers.

When an adenovirus vector infects a cell, it enters the cell by a process of receptor-mediated endocytosis.[47] Adenoviruses have an innate ability to escape the endolysosomal pathway, resulting in very efficient translocation of the DNA to the nucleus with subsequent high level episomal expression of exogenous DNA. Adenovirus vectors have been shown to be efficient *in vitro* and *in vivo*.[45,46,48,49] However, adenoviruses have known immunologic properties that may potentially limit their usefulness as human gene transfer vectors.[42-44] Moreover, because of transient expression, repetitive dosing will be required with this vector.

Current Approach to Gene Therapy in the United States

Current approaches to gene therapy in the United States are focused on the use of adenovirus-mediated gene transfer. Key issues to be addressed for successful gene transfer studies in patients with CF include the following: (1) efficiency; (2) efficacy; and (3) safety.

Efficiency

A previous *in vitro* study by Johnson and colleagues[50] suggested that as few as 6 to 10% of CF airway epithelial cells must be corrected in order to restore normal Cl^- transport function to an entire epithelial sheet. Efficient gene transfer using adenovirus vectors has been reported *in vivo* in cotton rats.[49] However, *in vivo* adenovirus-mediated gene transfer to the airway epithelia of nonhuman primates tends to be of low efficiency and patchy.[51] Efficient gene transfer to human airway epithelia *in vitro* has also been reported.[46] As discussed below, published data from a clinical study in the nasal epithelium of patients with CF may offer some exciting insights into *in vivo* gene transfer of CFTR in patients with CF.[52]

Efficacy

The current working definition of efficacy is the physiologic demonstration of restoration of normal Cl^- transport in CF epithelia following transduction of the wild-type human CFTR. In a previous study, Rosenfeld and colleagues[49] reported correction of the CF Cl^- permeability defect in a CF pancreatic cell line *in vitro* and also *in vivo* CFTR protein expression in cotton rat airway epithelia. More recently, Rich and colleagues[46] have reported *in vitro* correction of the Cl^- permeability defect in human CF airway epithelia with a multiplicity of infection (MOI) or number of infectious particles per cell as low as 0.1 to 1 using adenovirus-mediated transduction of CFTR.

To date, the mouse models for CF have not been used extensively in preclinical testing of adenovirus vectors. However, Grubb and colleagues (unpublished data, 1994) have demonstrated partial correction of the Cl^- transport defect in the nasal epithelium of their CF mouse model[53,54] following *in vivo* adenovirus-mediated gene transfer to the nasal epithelium. Interestingly, a high MOI was required to partially restore Cl^- transport. Moreover, no correction of sodium transport was observed. Recent evidence suggests that the results observed in the nasal studies of Grubb and colleagues may differ from observations in ongoing studies in patients with CF. In a series of three patients, Zabner and colleagues[52] have reported correction of Cl^- transport function in the nasal epithelium of subjects enrolled in their clinical study of adenovirus-mediated gene transfer for CF at a MOI as low as 1.

Safety

Several safety concerns have been raised with adenovirus vectors, including contamination of virus stocks with recombinant replication competent virus, immune response, ectopic expression, and integration. Contamination of virus stocks can be minimized by screening for replication competent viruses. Perhaps a more worrisome concern is whether the presence of E1 genes in the affected epithelium of patients with CF, which has been reported to be as high as 10 to 15%,[55] will lead to replication of adenovirus vectors in the host. For this reason, current human protocols evaluating safety and efficacy in patients have focused on the use of patients carrying antibodies to adenoviruses serotypes 2 or 5.

Wild-type adenoviruses are known to cause both an acute mixed cellular inflammatory response and a later lymphocyte predominant inflammatory response in rodents.[43,44] The acute response may be cytokine-mediated, while the late response is probably mediated by cytotoxic T cells.[43,44] Similarly, Engelhardt et al[51] and Trapnell et al[56] have been able to demonstrate both an acute neutrophil response and a late lymphocyte predominant inflammatory response in cotton rat and the baboon lung following direct delivery of adenovirus serotype 5-based vectors to the airways. Interestingly, the inflammatory responses were seen only when high doses of adenovirus vector were delivered to the alveolar region of the lung. In unpublished studies, we have seen similar dose-related inflammatory responses following adenovirus-mediated gene transfer to murine lungs (D. Thomas et al, unpublished results, 1994). These results suggest that the therapeutic to toxic ratio with adenovirus vectors may be small.

The data with regard to toxicity following ectopic expression of CFTR are mixed. Adenovirus-mediated gene transfer may lead to high-level expression in many different cell types in the airways. In a recent study by Stutts and colleagues,[57] clonal fibroblast cell lines overexpressing CFTR grew more slowly and had different electrophysiologic properties, *ie*, depolarized membrane potentials, when compared with fibroblasts that expressed CFTR at low levels or not at all. In contrast, transgenic mice that overexpress CFTR do not differ from control mice that do not overexpress the gene in their lung development, somatic growth characteristics, or reproductive function.[58] Hence, overexpression of CFTR may be manifested primarily in cells of nonepithelial origin that lack distinct apical and basolateral domains.

Adenovirus serotypes 2 and 5 have been used to efficiently transform human and rodent cell lines at frequencies up to 100-fold greater than obtained using calcium phosphate.[48] In general, transformation has been mediated through the E1 region genes, although substitution of simian virus 40 (SV40) sequences for the E1 genes will also enable stable transformation. The reports of E1 sequences present in the airway epithelial cellular genomes of CF and normal individuals[55] suggest that stable integration may also occur in airway epithelia. In preliminary *in vitro* studies from our laboratory, integration may occur in airway cells following adenovirus-mediated gene transfer at frequencies of up to 5 in 10,000 (J. Olsen et al, unpublished data, 1994). Given the requirement for repetitive dosing with *in vivo* adenovirus-mediated gene transfer of CFTR, the risk of insertional mutagenesis may be considerable.

Human safety and efficacy studies are currently underway at five CF centers. These studies are using low doses of adenovirus vectors in small numbers of patients with CF. If no toxicity is seen at the lower doses, the doses will be increased at subsequent stages of these trials. The initial results reported from the trial at the University of Iowa appear to be promising. One severe adverse reaction, that may have been cytokine-mediated, has been reported in one study involving the lower airways.

Conclusions

Major research efforts have been conducted to develop suitable gene transfer vectors for CF. Although many of these vector systems are still under development and are in need of improvement, initial clinical safety and efficacy trials for gene therapy of CF have begun. Liposome-mediated gene transfer has been the vector system selected for initial clinical studies in the United Kingdom, while clinical safety and efficacy trials in the United States all utilize adenovirus-mediated gene transfer. In this article, we have shown that adenovirus-mediated gene transfer to the airway epithelium can be efficient *in vivo*, although it may be rather inhomogeneous. Moreover, this method of gene transfer may be efficacious for CF based on evidence from mouse models and ongoing *in vivo* studies in humans. However, lung inflammation is a major concern with the use of high doses of adenovirus vectors. Moreover, the therapeutic to toxic ratio with repetitive dosing has not been defined. If gene therapy for CF is to become a reality, then continued development of more efficient vectors with improved safety profiles will be required.

References

1 Boat TF, Welsh MJ, Beaudet AL. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. The metabolic basis of inherited disease. New York: McGraw Hill, 1989; 2649-80

2 Quinton P. Cystic fibrosis: a disease in electrolyte transport.

FASEB J 1990; 4:2709-17
3 Rommens JM, Iannuzzi MC, Kerem BS, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989; 245:1059-65
4 Riordan JR, Rommens JM, Kerem BS, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245:1066-73
5 Cheng SH, Gregory RJ, Marshall J, et al. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 1990; 63:827-34
6 Anderson MP, Gregory RJ, Thompson S, et al. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. Science 1991; 253:202-05
7 Bear CE, Duguay F, Naismith AL, et al. Cl^- channel activity in Xenopus oocytes expressing the cystic fibrosis gene. J Biol Chem 1991; 266:19142-45
8 Kartner N, Hanrahan JW, Jensen TJ, et al. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. Cell 1991; 64:681-91
9 Drumm ML, Pope HA, Cliff WH, et al. Correction of the cystic fibrosis defect *in vitro* by retrovirus-mediated gene transfer. Cell 1990; 62:1227-33
10 Rich DP, Anderson MP, Gregory RJ, et al. Expression of the cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature 1990; 347:358-63
11 Olsen JC, Johnson LG, Stutts MJ, et al. Correction of the apical membrane chloride permeability defect in polarized cystic fibrosis airway epithelia following retroviral-mediated gene transfer. Hum Gene Ther 1992; 3:253-66
12 Kessler DA, Siegel JP, Noguchi PD, et al. Regulation of somatic-cell therapy and gene therapy by the Food and Drug Administration. N Engl J Med 1993; 329:1169-73
13 Miller AD. Human gene therapy comes of age. Nature 1992; 357:455-60
14 Miller AD, Rosman GJ. Improved retroviral vectors for gene transfer and expression. Biotechniques 1989; 7:980-90
15 Miller DG, Adam MA, Miller AD. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 1990; 10:4239-42
16 Leigh M, Kylander J, Yankaskas JR, et al. Cell proliferation in bronchial epithelium and submucosal glands of CF patients. Pediatr Pulmonol 1993; 9(suppl):251-52
17 Blacklow NR, Hoggan MD, Kapikian AZ, et al. Epidemiology of adeno-associated virus infection in a nursery population. Am J Epidemiol 1968; 88:368-78
18 Carter BJ. Parvoviruses as vectors. In: Tijssen PL, ed. Handbook of parvoviruses (vol 2). Boca Raton, Fla: CRC Press, 1990; 247-84
19 Flotte TR, Afione SA, Solow R, et al. Expression of the cystic fibrosis transmembrane conductance regulator from a novel adeno-associated virus promoter. J Biol Chem 1993; 268:3781-90
20 Egan M, Flotte T, Afione S, et al. Defective regulation of outwardly rectifying Cl^- channels by protein kinase A corrected by insertion of CFTR. Nature 1992; 358:581-84
21 Kotin RM, Menninger JC, Ward DC, et al. Mapping and direct visualization of a region-specific viral integration site on chromosome 19q-13qter. Genomics 1991; 10:831-34
22 Kearns W, Afione S, Cutting G, et al. AAV-CFTR vectors integrate at multiple chromosomal sites in a CF bronchial epithelial cell line [abstract]. Pediatr Pulmonol 1993; 9(suppl):242
23 Curiel DT, Agarwal S, Romer MU, et al. Gene transfer to respiratory epithelial cells via the receptor-mediated endocytosis pathway. Am J Respir Cell Mol Biol 1992; 6:247-52
24 Ferkol T, Kaetzel C, Davis PB. Gene transfer into respiratory epithelial cells by targeting the polyumeric immunoglobulin receptor. J Clin Invest 1993; 92:2394-2400
25 Ferkol T, Kaetzel C, Perales JC, et al. Gene transfer into respiratory epithelial cells using receptor-mediated endocytosis. Pediatr Pulmonol 1993; 9(suppl):77-8
26 Legendre JY, Szoka FC Jr. Delivery of plasmid DNA into mammalian cell lines using pH-sensitive liposomes: comparison with cationic liposomes. Pharm Res 1992; 9:1235-42
27 Gershon H, Ghirlando R, Guttman SB, et al. Mode of formation and structural features of DNA-cationic liposome complexes used for transfection. Biochemistry 1993; 32:7143-51
28 Debs R, Pian M, Gaensler K, et al. Prolonged transgene expression in rodent lung cells. Am J Respir Cell Mol Biol 1992; 7:406-13
29 Jarnagin WR, Debs RJ, Wang SS, et al. Cationic lipid-mediated transfection of liver cells in primary culture. Nucleic Acids Res 1992; 20:4205-11
30 Felgner PL, Gadek TR, Holm M, et al. Lipofection: a highly efficient, lipid-mediated DNA transfection procedure. Proc Natl Acad Sci USA 1987; 84:7413-17
31 Lu L, Zeitlin PL, Guggino WB, et al. Gene transfer by lipofection in rabbit and human secretory epithelial cells. Pflugers Arch 1989; 415:198-203
32 Brigham KL, Meyrick B, Christman B, et al. *In vivo* transfection of murine lungs with a functioning prokaryotic gene using a liposome vehicle. Am J Med Sci 1989; 298:278-81
33 Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle *in vivo*. Science 1990; 247:1465-68
34 Stribling R, Brunette E, Liggitt D, et al. Aerosol gene delivery *in vivo*. Proc Natl Acad Sci USA 1992; 89:11277-81
35 Hazinski TA, Ladd PA, DeMatteo CA. Localization and induced expression of fusion genes in the rat lung. Am J Respir Cell Mol Biol 1991; 4:206-09
36 Yoshimura K, Rosenfeld MA, Nakamura H, et al. Expression of the human cystic fibrosis transmembrane conductance regulator gene in the mouse lung after *in vivo* intratracheal plasmid-mediated gene transfer. Nucleic Acids Res 1992; 20:3233-40
37 Hyde SC, Gill DR, Higgins CF, et al. Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy. Nature 1993; 362:250-55
38 Alton EWFW, Middleton PG, Caplen NJ, et al. Non-invasive liposome-mediated gene delivery can correct the ion transport defect in the cystic fibrosis mutant mice. Nature Genet 1993; 5:135-42
39 Thomas DA, Myers MA, Wichert B, et al. Acute effects of liposome aerosol inhalation on pulmonary function in healthy human volunteers. Chest 1991; 99:1268-70
40 Stewart MJ, Plautz GE, Del Buono L, et al. Gene transfer *in vivo* with DNA-liposome complexes: safety and acute toxicity in mice. Hum Gene Ther 1992; 3:267-75
41 Nabel EG, Gordon D, Yang ZY, et al. Gene transfer *in vivo* with DNA-liposome complexes: lack of autoimmunity and gonadal localization. Hum Gene Ther 1992; 3:649-56
42 Berkner KL. Development of adenovirus vectors for the expression of heterologous genes. Biotechniques 1988; 6:616-29
43 Ginsberg HS, Moldawer LL, Sehgal PB, et al. A mouse model for investigating the molecular pathogenesis of adenovirus pneumonia. Proc Natl Acad Sci USA 1991; 88:1651-55
44 Ginsberg HS, Lundholm-Beauchamp U, Horwood RL, et al. Role of early region 3 (E3) in pathogenesis of adenovirus disease. Proc Natl Acad Sci USA 1989; 86:3826-27
45 Engelhardt JF, Yang Y, Stratford-Perricaudet LD, et al. Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenoviruses. Nat Genet 1993; 4:27-34
46 Rich DP, Couture LA, Cardoza LM, et al. Development and analysis of recombinant adenoviruses for gene therapy of cystic fibrosis. Hum Gene Ther 1993; 4:461-76